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Single tube single-color analysis of rifampicin and isoniazid allelic variants in MDR-TB

L. Wangh*, J. Rice, K. Pierce

Brandeis University, Waltham, MA, USA

Background: We have constructed a highly informative, rapid, single-tube, single-color assay for distinguishing variants in the *inhA* promotor, and *katG* and *rpoB* gene targets responsible for isoniazid and rifampicin resistance, using technologies invented at Brandeis University.

Methods & Materials: A multiplex LATE-PCR reaction is used to generate single-stranded DNA products for the Rifampicin Resistance Determining Region of the *rpoB* gene, the *katG* gene, and the *inhA* promotor, as well as an amplifiable internal control. These four amplicons are detected with four sets of Therma-Light probes all of which are labeled with Black Hole Quenchers with/without Quasar 670 fluorophors. Probe-target hybridization takes place at end-point over a wide range of temperatures below the reaction annealing temperature. The temperature ranges used for the four sets of Therma-Light probes are deliberately overlapped, making it possible to maximize the amount of information generated in a single color. The amplifiable internal control generates a specific very low temperature signal and an additional non-amplifiable internal control generates a second signal at a specific high temperature. Together these controls can be used to calibrate each reaction. Another proprietary reagent is added to the reaction mixture to improve primer specificity.

Results: The present assay has been tested on twenty-two different archival strains which are reported to harbor many of the common alleles responsible for resistance to rifampin and isoniazid. Each strain displayed its own "fluorescent signature" reflecting the underlying alleles present in the three target sequences. But, not every fluorescent signature agreed with archival information on the mutational composition of the strain. These discrepancies were resolved by sequencing the reaction products. The results show that the fluorescent signatures consistently identify the alleles correctly. Many more strains of *M. tuberculosis* will soon be tested.

Conclusion: This single color multiplex assay has both clinical and research applications and is just one of many possible assays for M(X)DR-TB that can be constructed using these technologies. For instance, Therma-Light probes labeled with fluorophores in other colors can be added for simultaneous analysis variants responsible for resistance to all other first and second line antibiotics.

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Evaluating a minipool strategy to screen for virologic failure and antiretroviral drug resistance

H.M. Newman[1,*], G. van Zyl[1], W. Preiser[1], L. Breunig[2]

[1] *University of Stellenbosch/National Health Laboratory Service, Tygerberg, Cape Town, South Africa*
[2] *University of Würzburg, Würzburg, Germany*

Background: For patients on antiretroviral treatment (ART), monitoring of HIV viral load is superior to clinical monitoring alone or clinical combined with CD4 count monitoring. However, the high cost of commercial viral load tests limits its use in resource-constrained settings. Commercial genotypic antiretroviral resistance testing is even more costly, yet it provides important additional information. Information on the presence of drug resistance mutations allows for an informed decision to be made regarding switching to second-line ART and which drugs to switch to, all of which could save money and improve patient outcomes. The novel strategy presented here, consisting of pooled testing by qualitative PCR with a defined threshold and limited sequencing of positive samples, provides a potential solution to this problem, allowing for cheaper, more efficient monitoring of patients on ART in resource-limited settings.

Methods & Materials: A total of 300 routine patient samples were included and tested in 60 pools of 5 samples each. A qualitative nested PCR using previously described primers was optimised for testing pools and individual samples from positive pools. All positive samples were tested for drug resistance-associated mutations. The results obtained by conventional viral load monitoring and by the pooling algorithm were compared.

Results: Twenty-two of sixty pools tested positive. Deconvolution of the positive pools yielded 29 positive individual samples. Twenty-six patients had viral loads of above 1000 copies per milliliter and were therefore failing therapy according to national criteria. The pooling algorithm detected 24 of those 26 patients, resulting in a negative predictive value of 99.3%. The sensitivity for detecting patients failing therapy was 92%. The commonest NRTI mutations were M184 V/I and K65R, with 7% and 2%, respectively, of the study population harboring these mutations. The commonest NNRTI mutation was K103N.

Conclusion: The pooled testing algorithm presented here was able to detect 24 of 26 adult patients failing first-line ART, and required 43% fewer assays than conventional viral load testing. In addition it provided drug resistance information, which is not routinely available in resourced-limited settings, and yielded a potential cost saving over individual viral load testing.

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